Captopril Ameliorates Myocarditis in Acute Experimental Chagas Disease

Juan S. Leon, BA; Kegiang Wang, MD; David M. Engman, MD, PhD

**Background**—Captopril, an angiotensin-converting enzyme inhibitor, is commonly prescribed to patients with Chagas heart disease (CHD). There are few human studies and no animal studies on the effects of captopril in CHD. We investigated the effects of captopril on myocarditis and the host immune response to *Trypanosoma cruzi* in an experimental model of acute CHD.

**Methods and Results**—A/J mice infected with Brazil strain of *T. cruzi* developed acute myocarditis by day 21 after infection, consisting of severe focal inflammation, necrosis, fibrosis, and *T. cruzi* pseudocysts. Administration of captopril (5 mg/L in the water) significantly reduced necrosis and fibrosis in infected mice. Increasing the captopril dose also led to a decrease in inflammation. Captopril did not affect overall mortality but did delay death while having no effect on parasitemia or cardiac parasite load. Treatment did not affect humoral immunity against *T. cruzi* or cardiac myosin (autoimmunity) but did decrease delayed-type hypersensitivity responses against both antigens. Interestingly, increasing the dose of captopril induced mortality in infected mice in a dose-dependent manner. Mortality was apparently not due to *T. cruzi* because neither parasitemia nor cardiac parasitosis was affected. The combination of captopril and infection may have impaired renal function because these mice had increased water consumption, decreased body mass, and increased serum BUN/creatinine ratio.

**Conclusions**—Captopril ameliorates the myocarditis associated with acute *T. cruzi* infection. *(Circulation. 2003;107:2264-2269.)*

**Key Words:** myocarditis ■ angiotensin ■ infection ■ collagen ■ myosin

*C. hagae* heart disease (CHD), caused by the protozoan *Trypanosoma cruzi*, is a significant cause of morbidity and mortality in South and Central America. Sixteen to eighteen million people are infected, and 120 million people are at risk of infection.¹ CHD is a potentially fatal dilated cardiomyopathy that develops in ≈30% of *T. cruzi*-infected individuals. Treatment of clinical CHD is similar to that of other cardiomyopathies, and includes sodium restriction and treatment with digitalis, diuretics, and angiotensin-converting enzyme (ACE) inhibitors, such as captopril and enalapril.² Captopril binds to the peptide-binding pocket of ACE and inhibits ACE’s catalytic production of angiotensin II; this also promotes an increase in the level of bradykinin. By interfering with the angiotensin II and bradykinin pathways, captopril reduces systemic arterial pressure, peripheral vascular resistance, and cardiac filling pressure, and increases cardiac output. Captopril is also an antiinflammatory agent, acting through the immunomodulatory actions of angiotensin II and the downstream effects of bradykinin (reviewed in Godsel et al³). Together, the effects of captopril result in reduced inflammation and fibrosis, improvement of cardiac function, and enhanced survival in heart failure patients.

Despite routine administration of captopril to patients with CHD, few studies have examined the effects of this drug on these individuals. Captopril has been shown to improve cardiac function with few side effects⁴,⁵ but has not been found to reduce mortality in CHD.⁶ Of concern is the report showing that in vitro administration of captopril enhances *T. cruzi* invasion of tissue culture cells by blocking the degradation of bradykinin.⁷ To date, however, there is no evidence that captopril increases *T. cruzi* parasitosis in humans. Captopril increases bacteremia in *Pseudomonas aeruginosa*-infected mice⁸ and elevated bradykinin is associated with bacteremia and mortality in *Vibrio vulnificus*-infected mice.⁹ Finally, prolonged captopril therapy for 6 months in a mouse model of Coxsackie viral myocarditis reduced myocardial fibrosis; however, mortality was increased for unknown reasons.¹⁰ Whether this is relevant to *T. cruzi* infection remains to be determined.

To address these issues, we ascertained whether administration of captopril to *T. cruzi*-infected mice would affect myocarditis or the host immune response to infection. The results provide compelling evidence that captopril ameliorates acute experimental CHD without affecting parasite load.
Methods

Mice and T cruzi
Four- to six-week-old male A/J mice (Jackson Laboratories, Bar Harbor, Maine) were housed under specific pathogen-free conditions. Mice were infected by intraperitoneal injection of \(1 \times 10^5\) Brazil strain T. cruzi trypomastigotes derived from infection of tissue culture H9C2 rat myoblasts (American Type Culture Collection). Parasitemia was measured from tailbleeds on a hemocytometer. Uninfected controls received an intraperitoneal injection of Dulbecco's Phosphate Buffered Saline (GibcoBRL) of equal volume. Mice were anesthetized by a single intraperitoneal injection of 60 mg/kg sodium pentobarbital for each experimental manipulation. The use and care of mice were conducted in accordance with the guidelines of the Center for Comparative Medicine (Northwestern University).

Captopril Regimen
Captopril was a gift from Dr. Agostino Molteni (Northwestern University, Chicago, Ill). Mice were given captopril at the indicated concentrations in their drinking water from initial infection to day of euthanasia. The captopril solution was changed 3 times a week and prepared fresh from powder every time.

Preparation of Cardiac Myosin and T cruzi Antigen
Cardiac myosin heavy chains were purified according to the method of Shiverick et al., with modifications as described. T. cruzi antigen was prepared from T. cruzi epimastigotes as described.

Histopathology
Hearts were removed, rinsed with PBS, and fixed for 24 hours in 10% buffered formalin. Fixed hearts were embedded in paraffin, sectioned, stained with hematoxylin-eosin or Masson's trichrome, and examined by light microscopy. Two sections were taken from each heart, one including both atria and the other both ventricles. Each section was examined for evidence of mononuclear and polymonuclear cellular inflammation, necrosis and mineralization, T. cruzi pseudocysts, and fibrosis and was assigned a histological score between 0 (no involvement noted) to 4 (100% involvement), with 1, 2, and 3 representing 25%, 50%, and 75% involvement of the histological section. Independent observers obtained substantial agreement on the scoring by weighted Kappa statistic as measured on a representative sample of 20 heart sections: overall agreement (0.82), inflammation (0.80), necrosis (0.96), pseudocysts (0.86), and fibrosis (0.92).

Serological Analysis
Levels of cardiac myosin-specific and T. cruzi-specific IgG were determined by ELISA as described. End-point dilution titers for total IgG were defined as the highest serum dilution that resulted in an absorbance value (OD450) of two standard deviations above the mean of a negative control (pooled sera from uninfected mice) included in every plate.

Delayed-Type Hypersensitivity
Myosin- and T cruzi-specific delayed-type hypersensitivity (DTH) was quantified using a standard ear swelling assay. Antigen-induced ear swelling was the result of mononuclear cell infiltration and exhibited typical DTH kinetics (ie, minimal swelling at 4 hours, maximal swelling at 24 to 48 hours after injection).

Clinical Chemistry
Serum levels of blood urea nitrogen (BUN), creatinine, and potassium were measured by the Center for Comparative Medicine (Northwestern University) according to standard methods.

Statistical Analyses
The statistical significance of DTH, blood chemistry, parasitemia, or log-transformed (base 2) antibody titers were analyzed by 1-way ANOVA followed by a 2-tailed \(t\) test and post hoc Bonferroni analysis. Comparison of histological scores was analyzed by the Pearson’s \(\chi^2\) test and post hoc Bonferroni analysis, and agreement of blinded observer histopathology scores was analyzed by weighted Kappa. Histopathological analysis was conducted on hearts of both expired and euthanized mice unless otherwise stated. Significant differences in mortality were analyzed by log rank test. Values of \(P < 0.05\) were considered significant.

Results
Captopril Treatment of T cruzi–Infected A/J Mice Decreases Cardiac Necrosis and Fibrosis
To investigate the effects of captopril on T. cruzi–induced myocarditis, we administered the drug to infected A/J mice in their drinking water (5 mg/L). Twenty-five days after infection, analysis of cardiac histopathology (Figure 1) revealed a significant decrease in necrosis and fibrosis in captopril-treated mice compared with untreated controls (Table 1). The incidence of myocarditis (all treated and untreated mice developed disease), body weight, heart weight, and heart weight to body weight ratio (Table 2) were not affected by
Captopril Administration Suppresses DTH but Not Humoral Immune Responses in Infected Mice

We investigated whether the antiinflammatory properties of captopril suppressed the immune response by assaying DTH and antibody production to *T. cruzi* and cardiac myosin (autoimmunity). *T. cruzi* DTH and myosin DTH were significantly lower in captopril-treated infected mice than in untreated controls (Figure 2A). Interestingly, T-cell proliferative responses to *T. cruzi* (not shown) and levels of *T. cruzi*-specific IgG and myosin-specific IgG were not affected by captopril (Figure 2B). Both *T. cruzi*-specific and myosin-specific IgM and IgG isotypes were also not affected by drug treatment (data not shown).

Captopril Administration Does Not Affect Parasitemia, Cardiac Parasite Tissue Load, or Mortality in Infected Mice

We tested whether captopril affects host susceptibility to infection by assessing mortality and parasite levels in treated and untreated mice. Captopril (5 mg/L in the water) did not affect mortality (Figure 3A), parasitemia (Figure 3B), or cardiac parasitosis (Table 1). In 3 separate experiments, captopril did, however, significantly delay death by 4 days (*P*<0.05).

Increasing the Dose of Captopril Decreases Cardiac Inflammation, Fibrosis, and Necrosis but Increases Mortality in Infected Mice

We hypothesized that higher doses of captopril might further reduce the severity of myocarditis. Administration of higher doses of captopril up to 75 mg/L decreased cardiac inflammation, fibrosis, and necrosis (Table 2). However, increasing the dose of captopril did not affect host susceptibility to infection as measured by mortality, parasitemia, or cardiac parasitosis (Table 1). The data suggest that the antiinflammatory properties of captopril may be dose-dependent, with lower doses providing protection against mortality but higher doses having no additional benefit.

### Table 1. Captopril Reduces Necrosis and Fibrosis in Infected Mice

<table>
<thead>
<tr>
<th>Captopril (5 mg/L)</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>−</td>
</tr>
<tr>
<td><strong>Necrosis</strong></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>−</td>
</tr>
<tr>
<td><strong>Parasite load</strong></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>−</td>
</tr>
<tr>
<td><strong>Fibrosis</strong></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>−</td>
</tr>
</tbody>
</table>

Values are No. of mice (% mice). Captopril-treated, saline-injected mice (n=10) and saline-injected mice (n=10) received scores of 0 for all parameters.

*P*<0.05 compared with untreated infected mice.

### Table 2. Captopril Does Not Affect the Heart Weight or Body Weight of Infected Mice

<table>
<thead>
<tr>
<th></th>
<th>Body, g</th>
<th>Heart, g</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected (n=30)</td>
<td>21.4±0.5*</td>
<td>0.122±0.006*</td>
<td>5.8×10⁻¹±3.0×10⁻⁴</td>
</tr>
<tr>
<td>Infected + captopril (n=30)</td>
<td>21.5±0.4*</td>
<td>0.115±0.005</td>
<td>5.6×10⁻¹±2.5×10⁻⁴</td>
</tr>
<tr>
<td>Uninfected (n=15)</td>
<td>23.6±0.5</td>
<td>0.107±0.002</td>
<td>4.6×10⁻¹±1.1×10⁻⁴</td>
</tr>
<tr>
<td>Uninfected + captopril (n=15)</td>
<td>23.8±0.6</td>
<td>0.106±0.003</td>
<td>4.4×10⁻¹±1.6×10⁻⁴</td>
</tr>
</tbody>
</table>

Results are expressed as the mean of mice in that group±SEM from pooling 3 separate experiments.

*P*<0.05 compared with respective uninfected controls.
mation, fibrosis, and necrosis in infected mice compared with untreated controls at 21 days after infection (Table 3), but did not further reduce the severity of myocarditis. Increasing the dose of captopril decreased body weight and heart weight in a dose-dependent manner, but had no significant effect on heart weight to body weight ratio (data not shown). Cardiac parasitosis was also not affected by increasing captopril dose, except for mice treated at 75 mg/L (Table 3). Restricting the analysis to mice that survived to 21 days after infection did not change these results. Mice administered 75 mg/L of captopril could not be analyzed in this manner because there were not enough mice for statistical analysis (n=3) in 3 separate experiments. Surprisingly, increasing the captopril dose also increased mortality in a dose-dependent manner (data not shown). The increased mortality was not solely due to direct captopril toxicity, however, because uninfected mice treated with 75 mg/L of captopril had no morbidity or mortality when examined out to 60 days after treatment. Mortality was also not due to enhanced parasite load because parasitemia (data not shown) and tissue parasitosis (Table 3) were not affected by increasing the captopril dose. Further analysis of these results revealed that increasing the dose of captopril led to increased water consumption and decreased body mass (data not shown), suggesting that renal function may be impaired in infected mice receiving higher doses of captopril. Supporting this idea, infected mice treated with 75 mg/L captopril exhibited a significant increase in the serum BUN/creatinine ratio compared with controls (Table 4). Infection also significantly increased the BUN/creatinine ratio compared with uninfected controls, whereas captopril without infection had no effect.

Discussion

We investigated the effect of captopril treatment on the outcome of T cruzi infection in mice. Captopril administration significantly decreased cardiac necrosis and fibrosis without affecting mortality or host parasite burden. Captopril decreased DTH to both T cruzi and cardiac myosin but had no effect on T-cell proliferative responses to T cruzi or IgG levels specific for either T cruzi or myosin. Finally, an increase in the captopril dose decreased necrosis, fibrosis, and inflammation, but also increased mortality.

These results are consistent with the few studies that showed an amelioration of cardiac function in Chagas pa-

<table>
<thead>
<tr>
<th>Captopril, mg/L</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>0 (0)</td>
<td>5 (25)</td>
<td>3 (15)</td>
<td>12 (60)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>0 (0)</td>
<td>3 (15)</td>
<td>10 (50)</td>
<td>7 (35)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>25*</td>
<td>0 (0)</td>
<td>2 (10)</td>
<td>13 (65)</td>
<td>5 (25)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>50*</td>
<td>0 (0)</td>
<td>4 (20)</td>
<td>15 (75)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>75*</td>
<td>1 (5)</td>
<td>8 (40)</td>
<td>11 (55)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>Necrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>0 (0)</td>
<td>8 (40)</td>
<td>12 (60)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>5*</td>
<td>10 (50)</td>
<td>10 (50)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>25*</td>
<td>5 (25)</td>
<td>15 (75)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>50*</td>
<td>5 (25)</td>
<td>14 (70)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>75*</td>
<td>8 (40)</td>
<td>12 (60)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>Parasitemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>1 (5)</td>
<td>14 (70)</td>
<td>5 (25)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>2 (10)</td>
<td>13 (65)</td>
<td>5 (25)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
<td>1 (5)</td>
<td>12 (60)</td>
<td>7 (35)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>50</td>
<td>1 (5)</td>
<td>19 (95)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>75*</td>
<td>16 (80)</td>
<td>4 (20)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>0 (0)</td>
<td>5 (25)</td>
<td>13 (65)</td>
<td>2 (10)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>1 (5)</td>
<td>12 (60)</td>
<td>7 (35)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>25*</td>
<td>2 (10)</td>
<td>13 (65)</td>
<td>5 (25)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>50*</td>
<td>3 (15)</td>
<td>16 (80)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
<tr>
<td>75*</td>
<td>6 (30)</td>
<td>12 (60)</td>
<td>2 (10)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>20</td>
</tr>
</tbody>
</table>

Values are No. of mice (% mice). Captopril-treated (75 mg/L), saline-injected mice (n=5) and saline-injected mice (n=5) received scores of 0 for all parameters.

*P<0.05 compared with untreated infected mice.
patients receiving captopril therapy and with results that showed a reduction in myocarditis on captopril treatment in other infectious myocarditides, including Coxsackie B and encephalomyocarditis virus infection. Captopril at 5 mg/L decreased fibrosis and necrosis but did not affect inflammation. Increasing the captopril dose significantly decreased inflammation (Table 3), in agreement with other studies of infectious myocarditis. Overall mortality was not affected by captopril at 5 mg/L (Figure 2A), although death was delayed by 4 days. We hypothesize that the delay in death is due to the antiinflammatory and antifibrotic effects of captopril, which may protect the heart (this study and Godsel et al). Lung, kidney, and other organs.

The potential concern that the antiinflammatory effects of captopril may render the host susceptible to T cruzi infection prompted us to investigate the effects of captopril on antigen-specific immunity. Captopril reduced T cruzi—and myosin-specific (autoimmune) DTH (Figure 2), but did not affect T cruzi–specific T-cell proliferation or T cruzi and myosin-specific IgG levels. Both myosin- and ovalbumin-immunized mice exhibit the same reduction in DTH and no effect on T-cell proliferative and B-cell responses on captopril treatment (L.M. Godsel, PhD, unpublished data, 2002). Captopril did not directly affect antigen-specific T- and B-cell function, because neither in vitro nor in vivo administered captopril directly affected antigen-specific T-cell proliferation or cytokine secretion (L.M. Godsel, PhD, unpublished data, 2002). Thus, the decrease in DTH may not be due to a direct effect on antigen-specific T-cell proliferation or cytokine secretion but may be due to a reduction in other important aspects of the inflammatory response, such as bradykinin production or T-cell trafficking (L.M. Godsel, PhD, unpublished data, 2002).

Captopril does not seem to affect either the host response or susceptibility to T cruzi. T cruzi parasitemia (Figure 3B) and tissue load were not affected by captopril at any dose (not shown and Table 3, respectively). These results are not consistent with a previous report showing enhanced T cruzi invasion of tissue culture cells on captopril treatment, perhaps because the host response is active at clearing T cruzi despite enhanced susceptibility to invasion or because captopril concentrations in vivo are too low to enhance invasion. Interestingly, captopril administration at 75 mg/L significantly reduced cardiac parasitosis, most likely because the dead mice included in the analysis did not survive long enough to have maximal cardiac parasitosis. Analysis of only living mice was not possible because these mice did not survive to a time point when histopathological analysis was reproducible.

Increasing the captopril dose also enhanced mortality in a dose-dependent manner, but did not enhance susceptibility to T cruzi (preceding paragraph). Captopril administration has also been shown to enhance mortality in Coxsackievirus–infected mice through an unknown mechanism. We do not know the precise cause of death in our mice. Mortality was not due to hyperkalemia induced by captopril (Table 4). It is possible that infection plus captopril administration impairs renal function in these mice because captopril stimulates water consumption, especially at higher doses, which in turn increases drug intake. The increase in BUN/creatinine ratios reflects impaired renal function, perhaps leading to dehydration and decreased body mass.

Taken together, these results suggest that captopril can reduce myocarditis and fibrosis without affecting host susceptibility to T cruzi infection. The mechanism of action of captopril could involve suppression of angiotensin II levels, enhancement of bradykinin levels, or a pharmacological effect of captopril thiol group, among other mechanisms. Antagonists of angiotensin II receptors reduced encephalomyocarditis virus–induced myocarditis. Enhanced bradykinin levels and activation of nitric oxide and prostaglandins by ACE inhibitors have been implicated in the reduction of infarct size, hypertrophy, and reduced collagen gene expression. Captopril’s cardioprotective effect may also be due to its upregulation of bradykinin, leading to nitric oxide synthesis, which may be important in resistance to acute T cruzi–induced myocarditis. Lastly, the thiol group of captopril is thought to ameliorate encephalomyocarditis virus–induced myocarditis by elimination of oxygen radicals. We are currently investigating these 3 possibilities.

Acknowledgments

This work was supported in part by grants from the US Public Health Service. J.S. Leon was supported by a predoctoral fellowship from the American Heart Association, Midwest Affiliate. We thank Dr A. Rademaker for advice on statistical analysis and Dr A. Molteni for the gift of captopril.

References


   Cardiology. 1994;84:261–267.

7. Scharfstein J, Schmitz V, Morandi V, et al. Host cell invasion by Trypanosoma cruzi is potentiated by activation of bradykinin B(2) receptors. 


14. Landis JR, Koch GG. The measurement of observer agreement for categorical data. 


20. Baba T, Kanda T, Kobayashi I. Reduction of cardiac endothelin-1 by angiotensin II type 1 receptor antagonist in viral myocarditis of mice. 


24. Baba T, Kanda T, Kobayashi I. Reduction of cardiac endothelin-1 by angiotensin II type 1 receptor antagonist in viral myocarditis of mice. 


30. Anning PB, Grocott-Mason RM, Lewis MJ. Effects of sulphhydryl- and non-sulphydryl-containing ACE inhibitors on left ventricular relaxation in the isolated guinea pig heart. 


Captopril Ameliorates Myocarditis in Acute Experimental Chagas Disease
Juan S. Leon, Kegiang Wang and David M. Engman

_Circulation_. 2003;107:2264-2269; originally published online April 21, 2003;
doi: 10.1161/01.CIR.0000062690.79456.D0
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/107/17/2264

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org/subscriptions/